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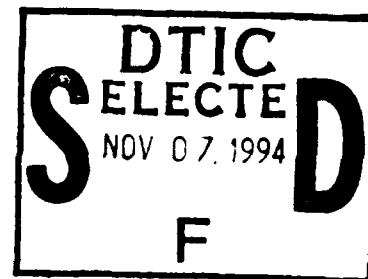
TITLE: THE 3D STRUCTURE OF STAPHYLOCOCCAL ENTEROTOXINS

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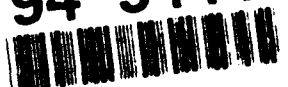


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October 15, 1994 Annual Report (10/1/93-9/30/94)

THE 3D STRUCTURE OF STAPHYLOCOCCAL ENTEROTOXINS

MIPR No.

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Martin Sax, J. Pletcher, S. Swaminathan

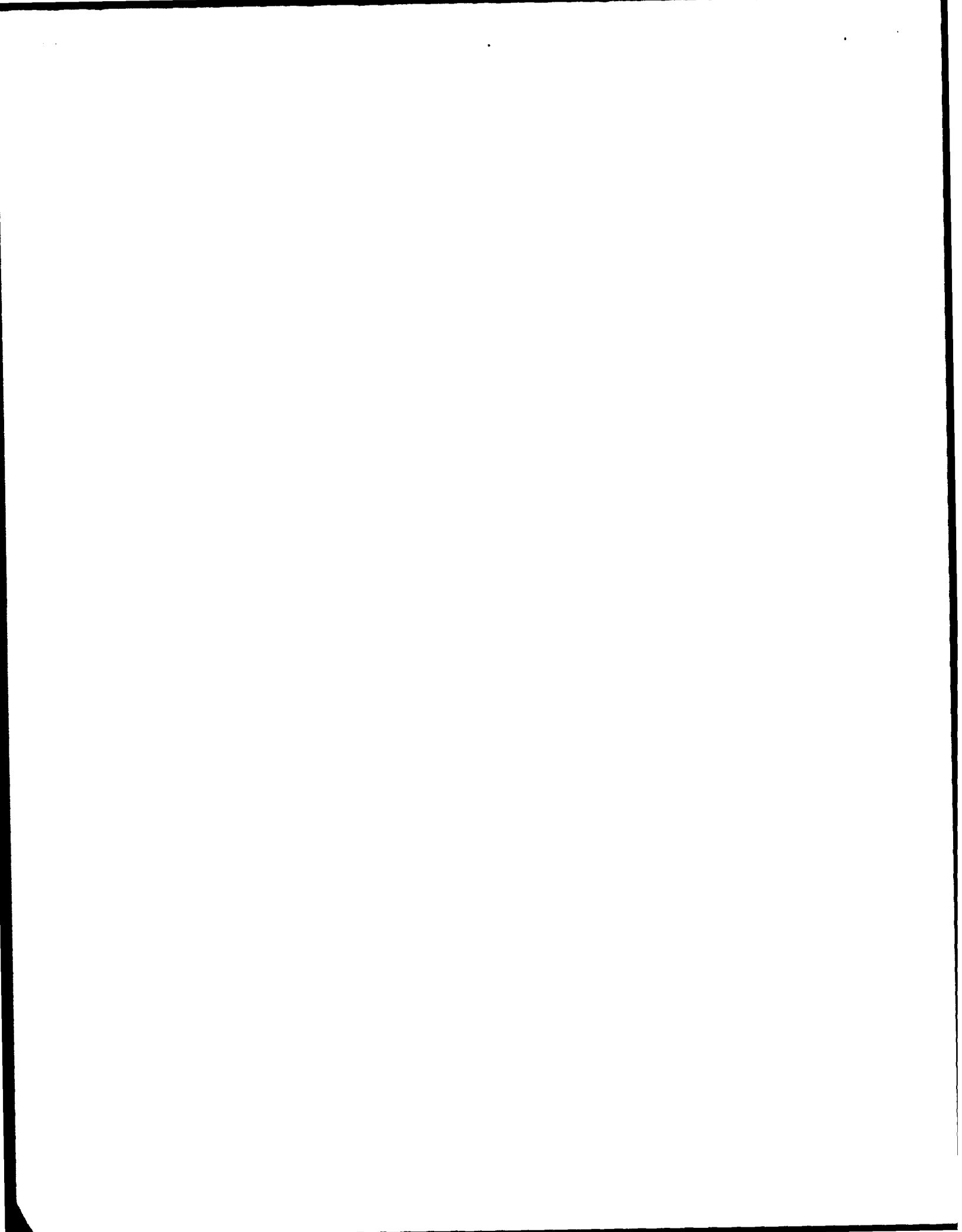
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The purpose of this research is to determine the crystal structure of the staphylococcal enterotoxins (SEs) by x-ray diffraction and protein modeling techniques and to correlate the 3D structures with their biological activities, in order to provide essential data for use in vaccine, or other effective counteractant, development. Several structures were determined in the project period covered by this report. These include the crystal structures of the following by x-ray diffraction: SEC2, form III of SEB, a mutant (F44 → S) of SEB, and a complex of SEB cocrystallized with lactose. Based on the concept that all SEs possess a common main chain folding motif, SEE and SEA were modeled from the empirical SEB structure.

Staphylococcal enterotoxins, crystal structure,  
superantigen, protein crystallography, x-ray diffraction



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## TABLE OF CONTENTS

Front Cover	1
SF 298	2
Foreword	3
Table of Contents	4
Introduction	5
Body	5
Conclusion	7
References	7
Figure	8

## **STAPHYLOCOCCAL ENTEROTOXINS - ANNUAL REPORT**

### **INTRODUCTION:**

Staphylococcal enterotoxins secreted by *staphylococcus aureus* cause diarrhea in humans. These toxins are responsible for about 25% of food poisoning cases in the United States of America. There are five distinct serotypes of s. enterotoxins labeled SEA through SEE. These are divided into two groups based on their sequence homology. SEA, SED, and SEE form one group while SEB and SEC form the other. SEC itself could be further subdivided into SEC1-3 depending on the epitope variation. S. enterotoxins have recently been identified as superantigens. These toxins when presented by major histocompatibility complex class II (MHCII) molecules can induce massive proliferation of polyclonal T cells bearing particular types of variable ( $V\beta$ ) chains by forming a ternary complex of MHCII, superantigen and T-cell receptor (TCR). Unlike ordinary processed antigens, intact superantigens bind to a wide variety of MHCII molecules but with different degree of affinity. Binding studies of s. enterotoxins to MHCII molecules have shown that different s. enterotoxins bind to different sites in MHCII and some at more than one place. Similarly s. enterotoxins within the same group have different  $V\beta$  specificities. Though a model has been proposed for the formation of the ternary complex, recent results indicate that the model could be different depending on the type of s. enterotoxin. The crystal structure of MHCII has revealed that its structure is very similar to the MHC class I molecule. Theoretical models of  $V\beta$  chains have been proposed based on the structures of immunoglobulins. The crystal structures of SEB, toxic shock syndrome toxin (TSST) and SEC3 have been determined. With these structures available it should be possible to model a ternary complex since no experimental model is available at the moment.

### **BODY:**

The crystal structure of SEB has been determined in this laboratory. The molecule consists of two domains (see figure 1); domain 1 is made up of a five stranded  $\beta$  cylinder with one end of the cylinder capped by a small  $\alpha$  helix ( $\alpha 3$ ). Domain 2 mainly consists of two  $\alpha$  helices with a five stranded twisted  $\beta$  sheet covering one side of both helices. Based on the topology of the molecule and on the results of mutational studies, regions and sites relevant for MHCII and TCR binding were proposed. The TCR binding site is located at the top of the molecule and is at the interface of the two domains. MHC binding site was proposed to be the entire front side of the molecule (on the side of  $\alpha 5$  helix). It was also proposed that in spite of their limited sequence homology all s. enterotoxins will possess a common folding pattern similar to SEB. This hypothesis has been proved to be right by the crystal structure determinations of TSST-1, SEC3 and SEC2 (discussion following). The three dimensional structure of SEB has helped scientists in designing experiments for

elucidating the mechanism of action of the enterotoxin and for identifying epitopes for developing vaccines (Jett et al., 1994).

The major aim of this project was to determine the crystal structure of all s.enterotoxins. In the past year we have crystallized SEC2 and determined its crystal structure. SEC2 was crystallized in two forms. Form 1 crystals were obtained using 20% PEG 6000, 0.1M Hepes at pH 7.0; form 2 crystals were obtained using 20% PEG 8000, 0.2M magnesium acetate and 0.1M cacodylate at pH 6.5. Form 1 crystals are in space group P2<sub>1</sub> with cell dimensions  $a = 43.43$ ,  $b = 69.92$ ,  $c = 42.22$  Å and  $\beta = 90.1^\circ$ . Form 2 crystals are in tetragonal space group P4<sub>1</sub>2<sub>1</sub>2 or P4<sub>3</sub>2<sub>1</sub>2 with cell dimensions  $a = b = 42.98$  and  $c = 289.92$  Å. Native diffraction data were collected from form 1 crystals using Siemens area detector mounted on a Rigaku rotating anode. Monochromatic Cu K $\alpha$  radiation was obtained using thin nickel foil and Franks double focusing mirrors. The data is 83% complete to 2.7 Å resolution. The structure was solved by the molecular replacement method using programs MERLOT and RMAP. The SEB molecule was used as a search model. Since MERLOT gave two solutions with the same orientation but different translations, a derivative data set was collected for a crystal soaked in a heavy atom reagent (platinum diamino dichloride). Heavy atom phases were used to identify the correct solution. The structure was refined by simulated annealing method using XPLOR. The bias in the model was checked and corrected using simulated annealing delete maps. The final R factor is 0.188 for 5174 reflections with  $I > 1.0\sigma(I)$  in the resolution range 10 - 2.7 Å. The RMSD in bond lengths and bond angles are 0.020 Å and 4.0° respectively. The overall structure of SEC2 is very similar to SEB. There are six differences in the amino acid sequence in the TCR binding site and the binding sites are being compared now. A paper on the crystallization and preliminary studies on SEC2 has been submitted to Acta Crystallographica Section D for publication. A detailed paper on the three dimensional structure of SEC2 is in preparation.

As reported in the last annual report, domain 1 of SEB has been identified as oligomer or oligonucleotide binding site (OB fold). SEB has been shown to bind to glycosphingolipids in kidney cells. We have determined the structure of SEB cocrystallized with lactose which is the head group of lactosylceramide. Lactose is bound to SEB but not exactly at the same site as reported for other toxins possessing the OB-fold; it appears to act as a cross link between SEB molecules.

Small crystals of SED have been obtained but these crystals diffract X-rays poorly. Crystals of SEB and 3'-N-acetylneuramin-lactose complex have also been obtained. This trisaccharide is the head group of another glycosphingolipid, GM3.

Based on the experimental evidence supporting our original hypothesis that all s.enterotoxins will have the same common fold we have modeled SEA and SEE using the three dimensional structure of SEB. These two structures are now being analyzed in conjunction with the experimentally determined structures of SEB and SEC2.

#### **CONCLUSION:**

1. Even though form 2 crystals SEC2 diffract to better than 1.9 Å resolution a good native diffraction data could not be collected with the in-house facility because of the long c-axis. Since the crystal structure determination of this form should provide finer details of the molecule, we are planning to collect a high resolution data with synchrotron facility at Brookhaven National laboratory, Upton, New York.

2. Efforts will be made to improve the quality of SED crystals. Crystallization of other s.enterotoxins will be continued.

3. Papers will be written up on the high resolution structure of SEB form III crystals and an SEB mutant with Phe 44 replaced by Ser (F44 → S).

4. The structure - function relationships in SEB which were described first by us (Swaminathan et al., 1992) were deduced from the 3 dimensional structure analysis of SEB and from the available mutational data. We now are extending the technique to other members of the SE family of proteins. The goal is to correlate structural differences with variations in the biological activities of the member proteins, in order to gain further precision in defining the stereochemical factors influencing their activities.

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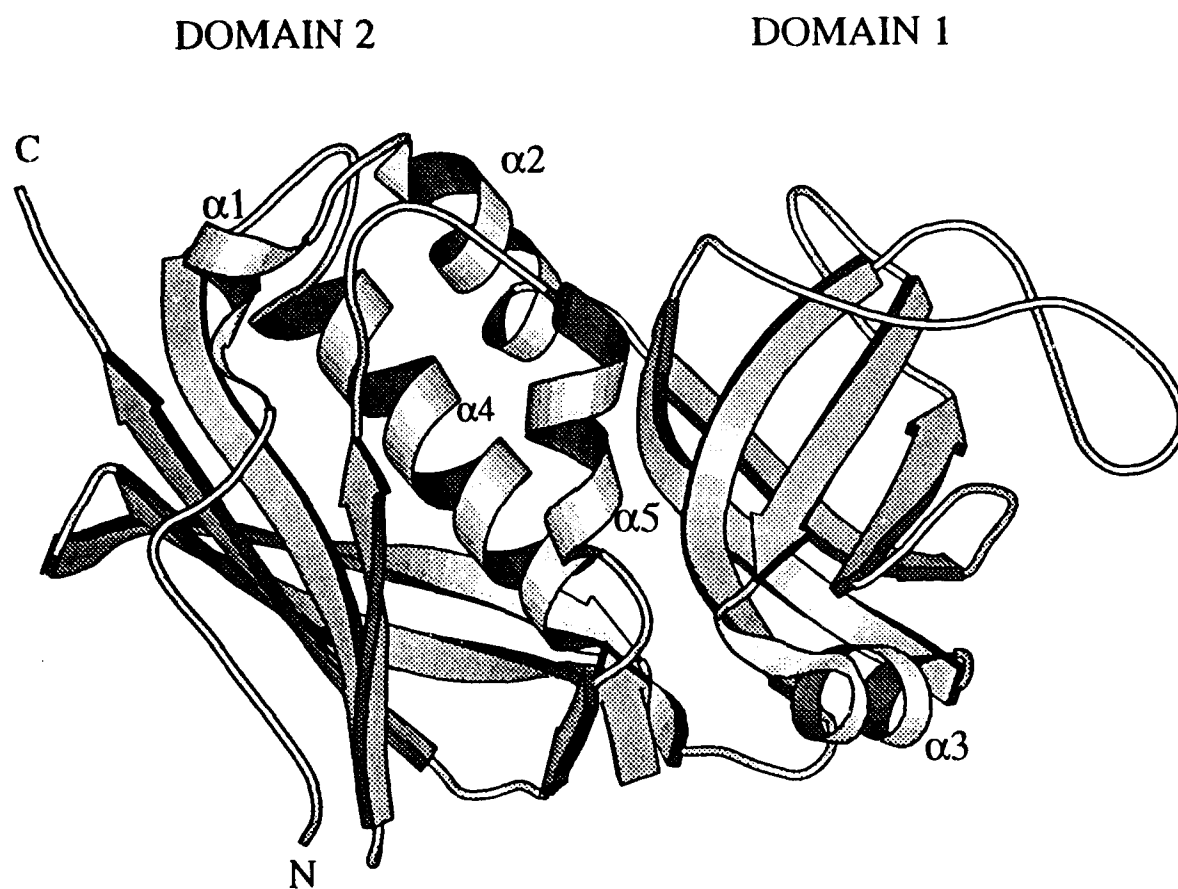


FIGURE 1: SEB